

Investigation of the Toxic & Teratogenic Effects of GRAS Substances to the  
Developing Chicken Embryo

2/28/74

AGAR GRACILIMR/4

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Date: February 28, 1974

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SUBJECT: Investigation of the Toxic and Teratogenic Effects of GRAS  
Substances to the Developing Chicken Embryo

Attached is the report of the investigation of AGAR  
GRACILARIA in the developing chicken embryo.

Investigations of the Toxic and Teratogenic Effects of  
GRAS Substances to the Developing Chicken Embryo:

AGAR GRACILARIA

PROTOCOL:

Agar gracilaria (1) was tested for toxic and teratogenic effects to the developing chicken embryo under four sets of conditions. It was administered, with water as the solvent, by two routes and at two stages of embryonic development; via the albumen at pre-incubation (0 hours) and at 96 hours of incubation, and via the yolk at 0 hours and at 96 hours using techniques that have been described previously (2, 3, 4).

The route of albumen, instead of the usual air cell, was chosen because of the fact that the administered agar gracilaria solution formed globular coagulates as soon as it was injected into the air cell, and absorption through the embryonic membrane was not conceivable.

Groups of ten or more eggs were treated under these four conditions at several dose levels until a suitable total number of eggs per level was reached for all levels allowing some to hatch. Groups of adequate size were treated solely with the solvent at corresponding volumes. Untreated controls were also included in each experiment.

After treatment, all the eggs were candled daily and the non-viable embryos were removed. Surviving embryos were allowed to hatch. Hatched chicks and non-viable embryos were examined grossly for abnormalities (internally and externally) as well as for toxic responses such as edema and hemorrhage. Along with these, histological examinations of major organs (liver, heart, kidney, lung, brain, intestine, gonad, and some endocrine organs) were carried out by taking samples from a representative number of animals from each experimental group.

RESULTS:

The results obtained are presented in Tables 1 through 4 for each of the four conditions of the test.

Columns 1 and 2 give the dose administered in milligrams per egg and milligrams per kilogram egg weight, respectively. (The milligrams per kilogram figure is based on an average egg weight of fifty grams.)

Column 3 is the total number of eggs treated.

Column 4 is the percent mortality, i. e. , the total number of non-viable eggs divided by the total number of treated eggs.

Column 5 is the total number of abnormal birds expressed as a percentage of the total number of eggs treated. This includes all the abnormalities observed and also the toxic responses such as edema, hemorrhage, hypopigmentation of the down and other disorders such as feather abnormalities, significant growth retardation, cachexia, and neural disorders including ataxia.

Column 6 is the total number of birds having a structural abnormality of the head, viscera, limbs, or body skeleton expressed as a percentage of the total number of eggs treated. Toxic responses and disorders such as those noted for column 5 are not included.

Columns 3 through 6 have been corrected for accidental deaths if any occurred. Included in these columns are comparable data for the solvent-treated eggs and the untreated controls.

The mortality data in column 4 have been examined for a linear relationship between the probit percent mortality versus the logarithm of the dose according to the procedures of Finney (5). The results obtained are indicated at the bottom of each table.

The data in columns 4, 5 and 6 have been analyzed using the Chi Square test for significant differences from the solvent background. Each dose level is compared to the solvent value and levels that show differences at the 5% level or lower are indicated by an asterisk in the table.

## DISCUSSION:

Agar gracilaria was found to be embryotoxic when administered to the embryos under all conditions of the test. The toxicity was significantly ( $P=0.05$ ) greater than solvent-treated eggs at all dose levels tested without exception. Probit analysis reveals an  $LC_{50}$  of 6.673 mg/egg (albumen at 0 hours, Table 1) and an  $LC_{50}$  of 5.997 mg/egg (albumen at 96 hours, Table 2). Yolk treatment at 0 hours resulted in an  $LC_{50}$  of 4.595 mg/egg and at 96 hours it was 2.479 mg/egg (Tables 3 and 4, respectively).

Abnormal birds were seen under all four conditions of the test, but the incidence of birds having a structural abnormality of the head, limbs, viscera, or skeleton was not significantly different from that of the solvent background ( $P=0.05$ ). Of the 142 control eggs, only one abnormal bird with curled toes was found.

ALBUMEN AT 0 HOURS: Abnormalities were found in all the dose levels at 0 hours. At 5.0 mg/egg, four abnormal birds were found with

Table 4  
Agar Gracilaria  
Yolk at 96 Hours

Dose		Number of eggs	Percent Mortality	Percent Abnormal	
mg/egg	mg/kg			Total	Structural
5.0	100	131	52.67*	3.81	2.29
1.0	20	131	52.67*	1.52	1.52
0.5	10	131	41.22*	1.52	1.52
0.25	5	131	17.55*	1.52	1.52
Water		131	3.05	0.76	0.76
Control		142	7.74	0.70	0.70

LC<sub>30</sub> 0.381 mg/egg (7.621 mg/kg)

LC<sub>50</sub> 2.479 mg/egg (49.584 mg/kg)

LC<sub>90</sub> 240.996 mg/egg (4819.929 mg/kg)

\*Significantly different from solvent  $P \leq 0.05$

Table 3  
Agar Gracilaria  
Yolk at 0 Hours

Dose		Number of eggs	Percent Mortality	Percent Abnormal	
mg/egg	mg/kg			Total	Structural
5.0	100	130	51.53*	3.84	3.07
1.0	20	130	43.84*	3.07	3.07
0.5	10	130	23.84*	2.30	1.53
0.25	5	130	22.30*	1.53	1.53
Water		130	6.15	0	0
Control		142	7.74	0.70	0.70

LC<sub>30</sub> 0.881 mg/egg (17.633 mg/kg)

LC<sub>50</sub> 4.595 mg/egg (91.900 mg/kg)

LC<sub>90</sub> 259.746 mg/egg (5194.921 mg/kg)

\*Significantly different from solvent  $P \leq 0.05$

Table 2

## Agar Gracilaria

## Albumen at 96 Hours

Dose		Number of eggs	Percent Mortality	Percent Abnormal	
mg/egg	mg/kg			Total	Structural
5.0	100	97	47.42*	3.09	2.06
1.0	20	97	35.05*	3.09	3.09
0.5	10	97	37.11*	3.09	1.03
0.25	5	97	14.43*	1.03	1.03
Water		127	5.51	0	0
Control		142	7.74	0.70	0.70

LC<sub>30</sub> 0.944 mg/egg (18.894 mg/kg)

LC<sub>50</sub> 5.997 mg/egg (119.956 mg/kg)

LC<sub>90</sub> 549.188 mg/egg (10983.773 mg/kg)

\*Significantly different from solvent  $P \leq 0.05$

Table 1  
Agar Gracilaria  
Albumen at 0 Hours

Dose		Number of eggs	Percent Mortality	Percent Abnormal	
mg/egg	mg/kg			Total	Structural
5.0	100	98	48.97*	5.10	4.08
1.0	20	98	32.65*	5.10	3.06
0.5	10	98	26.53*	3.06	3.06
0.25	5	98	19.38*	3.06	2.04
Water		128	6.25	1.56	1.56
Control		142	7.74	0.70	0.70

LC<sub>30</sub> 1.220 mg/egg (24.415 mg/kg)

LC<sub>50</sub> 6.673 mg/egg (133.477 mg/kg)

LC<sub>90</sub> 423.991 mg/egg (8479.835 mg/kg)

\*Significantly different from solvent  $P \leq 0.05$



1. Agar gracilaria, powdered, U.S.P. XVIII, FDA 71-60, Dr. Madis Laboratories, 375 Huyler Street, South Hackensack, New Jersey 07606, Lot No. 2-1353
2. McLaughlin, J., Jr., Marliac, J.-P., Verrett, M.J., Mutchler, M.K. and Fitzhugh, O.G. Toxicol. Appl. Pharmacol. 5:760-770, 1963
3. Verrett, M.J., Marliac, J.-P. and McLaughlin, J., Jr. JAOAC 47: 1002-1006, 1964
4. Hanan, E.B., Am. J. Anat. 38:423-450, 1927
5. Finney, D.J. Probit Analysis, 2nd ed., Cambridge Press, Cambridge, Appendix I, 1964

one of the following: dysgnathia, celosomia, hip contracture or agenesis of the foot. At 1.0 mg/egg, three abnormal birds, all with hip contractures, were seen. At 0.5 mg/egg, two birds with curled toes and one with celosomia were found. At 0.25 mg/egg, two birds were abnormal with curled toes. The solvent-treated controls produced two abnormal birds, both with curled toes.

ALBUMEN AT 96 HOURS: Abnormal birds were seen at all dose levels except for the solvent-treated controls where no abnormalities were produced. At 5.0 mg/egg, one bird had celosomia and the other had hip contracture. At 1.0 mg/egg, three abnormal birds were found, each with one of the following: hip contracture, curled toes, celosomia. At 0.5 mg/egg, only one abnormal bird, which had dysgnathia, was found. At 0.25 mg/egg, one bird with curled toes was seen.

YOLK AT 0 HOURS: The solvent-treated birds were the only group that did not produce any abnormalities. At 5.0 mg/egg, four abnormal birds, one with an abnormal curvature of the maxilla and three with hip contractures, were seen. At 1.0 mg/egg, a total of four birds were abnormal; one with celosomia, two with hip contractures and the fourth with curled toes. At 0.5 mg, one bird with celosomia and the other bird with hip contracture were found.

YOLK AT 96 HOURS: Abnormalities were seen at all tested levels. The solvent-treated birds produced only one abnormal bird with curled toes. At 5.0 mg/egg, four abnormal birds were found with one of the following: celosomia, abnormal curvature of the maxilla, hypopigmentation of the down, agenesis of the toes. At 1.0 mg/egg, one had hip contracture and the other had curled toes. At 0.5 mg/egg, two birds, one with celosomia and the other with hip contracture, were seen. At 0.25 mg/egg, one bird with hip contracture and the other bird with curled toes were observed.

Histological examinations of the major organs revealed no evidence of consistent change due to either the dose level of the substance administered or the mode of treatment.

From these observations, it is concluded that agar gracilaria is not particularly teratogenic. While the statistical result does not indicate the significance of the frequency of the incidence of the abnormalities, many abnormalities that were not seen in control or solvent-treated birds were observed. These include dysgnathia, abnormal maxillary curve and celosomia.